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Dr. William Stokes
Environmental Toxicology Program
National Institute of Environmental Health Sciences, MD WC-05
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Stokes:

I request that the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) organize a workshop to evaluate the Frog Embryo Teratogenicity Assay - *Xenopus* (FETAX). This 96 hour assay, developed to assess developmental toxicity, appears to meet many of the validation criteria recommended by ICCVAM, and it has been used in both human health and ecological assessments. Possible regulatory applications for human health evaluations for developmental toxicity include screening and prioritizing compounds for further testing, evaluating complex chemical mixtures in environmental samples, and as supplemental information in a weight of evidence evaluation of human developmental toxicity hazards. Some advantages and potential limitations of FETAX are listed in the attachment.

The proposed workshop should evaluate the validation status of FETAX based on ICCVAM criteria, the usefulness of FETAX for the potential regulatory uses, and the critical research, development, and validation efforts needed to improve the method. This workshop will be jointly sponsored by the National Center for Environmental Assessment, U.S. Environmental Protection Agency, and the Environmental Toxicology Program of the National Institute of Environmental Health Sciences.

William H. van der Schalie, Ph.D.
Ecologist

Attachment

Advantages and Limitations of the FETAX Method

Some advantages include:

- standardized test protocol (American Society for Testing and Materials method)
- published atlas of abnormalities
- evaluated against a data base of over 100 compounds, including known mammalian developmental toxicants as well as compounds not known to cause developmental toxicity; overall accuracy is approximately 90%
- some mechanistic data indicating similarities between developmental toxicity in FETAX, mammals, and humans
- can be used with or without a rat liver microsome metabolic activation system
- can be used either in the laboratory or in situ
- can evaluate single chemicals or complex mixtures

Possible limitations or areas requiring further discussion:

- Appropriateness of endpoint (teratogenicity index; LC50 divided by EC50 [malformations])
- when used with environmental samples or in an in situ test, understanding the influence of environmental parameters such as ionic strength, pH, dissolved oxygen, etc. on FETAX responses
- appropriate applications for regulatory purposes and interpretation of data for human health purposes

Overview of the FETAX Test System

FETAX (Frog Embryo Teratogenesis Assay-Xenopus) is a 96-hour whole embryo developmental toxicity test that utilizes embryos of the South African clawed frog, *Xenopus laevis*. The most important FETAX endpoints are the 96-h LC50, 96-h EC50 (malformation), the teratogenic index (found by dividing the LC50 by the EC50) and growth inhibition. Additional data concerning type and severity of malformation is also recorded. FETAX was initially designed as an indicator of potential human developmental health hazards and this use has been enhanced by the development of an in vitro metabolic activation system using Aroclor 1254- and Isoniazid-induced rat liver microsomes. FETAX has undergone extensive validation using single chemicals of known mammalian developmental toxicity and after testing >100 compounds the predictive accuracy to mammals is at least 90%. FETAX is also used extensively in ecotoxicology.

FETAX can be used in a variety of testing formats which include surface and groundwater aqueous samples in static, static-renewal and flow-through formats. The assay is robust enough to accommodate volatile and non-polar organics, soil and sediment samples and even pathogens.

FETAX can be used as a developmental toxicity assay to:

- 1) initially assess and prioritize compounds prior to mammalian testing.
- 2) examine structure-activity relationships in chemical classes.
- 3) investigate the biotransformation of developmental toxicants.
- 4) test complex environmental mixtures.
- 5) be used in the Toxicity Identification Evaluation process.
- 6) assess the health of aquatic ecosystems.
- 7) establish fresh water and sediment criteria.
- 8) in characterizing hazardous waste sites (e.g. Superfund sites).

An American Society for Testing and Materials New Standard Guide for the Conduct of FETAX was published along with a companion Atlas of Abnormalities that aids in embryo staging and identifying malformations.

As part of the ASTM process, a six-laboratory interlaboratory validation study (ILS) was conducted to determine the repeatability and reliability of FETAX. Secondary goals were the improvement of the FETAX protocol and the testing of additional compounds whose mammalian developmental toxicity was known. Excellent results were obtained even when the embryos were co-cultured with the metabolic activation system. FETAX is now ready to be evaluated as a viable alternative model.